Nature Conservation

Nature Conservation 59: 207–229 (2025)
DOI: 10.3897/natureconservation.59.152167

Research Article

DNA metabarcoding reveals distinct trophic niches among sympatric aerial insectivores (Family: Apodidae and Hirundinidae) in central Peninsular Malaysia

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Abstract

Understanding the dietary preferences of aerial insectivores is essential for assessing their ecological roles in ecosystem energy flow. Despite this importance, few integrative studies have examined dietary overlap and niche segregation among coexisting aerial insectivore species, particularly in human-modified mosaic landscapes. This study addresses that gap by analysing the diets of four aerial insectivorous bird species: Apodidae, the House Swift (*Apus nipalensis*), House-farmed Swiftlet (*Aerodramus* sp.), Hirundinidae, the Barn Swallow (*Hirundo rustica*), and Pacific Swallow (*Hirundo tahitica*), within an urban–agricultural forest interface in central Peninsular Malaysia. A total of 60 faecal samples were collected over a one-week period in March 2022 and analysed using high-throughput sequencing of the mitochondrial COI gene. The results revealed arthropod prey from 13 orders, 97 families, 93 genera, and 65 species. Common prey orders included Hymenoptera, Diptera, and Coleoptera. Dietary diversity was highest in *H. rustica* indicating a generalist foraging strategy, while *A. nipalensis* showed the lowest prey richness and a more specialised diet. The varied dietary patterns among sympatric species and the dietary diversity reflect trophic niche partitioning and highlight the functional role of aerial insectivores in natural pest regulation. Ongoing environmental changes, particularly urbanisation, habitat fragmentation, and declining insect populations due to pesticide use pose growing threats to aerial insectivores. These findings highlight the need to conserve insect-rich habitats within mixed-use landscapes and support the application of metabarcoding as a tool for monitoring ecosystem health and guiding biodiversity-friendly land-use planning.

Key words: Aerial insectivores, ANML primer sets, cytochrome c oxidase I (COI), dietary segregation, high throughput sequencing, mosaic landscape

OPEN ACCESS

Academic editor: Sze Huei Yek
Received: 6 March 2025
Accepted: 19 May 2025
Published: 20 August 2025

ZooBank: https://zoobank.org/29D110E0-8082-4043-8DF7-234C286E3FB2

Citation: Arazmi FN, Ismail NA, Daud UNS, Mansor MS (2025) DNA metabarcoding reveals distinct trophic niches among sympatric aerial insectivores (Family: Apodidae and Hirundinidae) in central Peninsular Malaysia. Nature Conservation 59: 207–229. https://doi.org/10.3897/natureconservation.59.152167

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Introduction

Arthropodivorous birds, which primarily consume arthropods such as insects and spiders (Lopes et al. 2016), play vital roles in maintaining the health and stability of ecosystems globally. Their ecological function extends beyond merely regulating insect populations to preserving environmental balance

which benefits both natural habitats and agricultural systems. These birds contribute to nutrient cycling by preying on insects and maintaining the stability of food chains by being both predators and prey (Sekercioglu 2006; Whelan et al. 2008). As predators, they serve as effective biological control agents by regulating herbivorous insect populations, particularly within agricultural ecosystems (Otieno and Mukasi 2023). Consequently, aerial insectivores are often considered indicator species (Beskardes 2020; Lourenço et al. 2021; Dunoyer et al. 2024), with their abundance and species richness reflecting the overall state of the ecosystem.

Aerial insectivores, especially birds that forage on flying insects, are often considered key indicators of ecosystem health and integrity due to their sensitivity to changes in insect availability, habitat quality, and environmental conditions (Spiller and Dettmers 2019). Understanding the feeding behaviour and dietary preferences of aerial insectivores is crucial for their conservation. They play a significant ecological role in environment as biological control of insect pests. Aerial insectivores, such as swallows (Hirundinidae), swifts and swiftlets (Apodidae), flycatchers (Muscicapidae) and nightjars (Caprimulgidae) exhibit diverse feeding adaptations and actively forage for insects while in flight (Morse 1971; Kardynal and Imlay 2022). Aerial insectivores are opportunistic predators with broad trophic niches (Orłowski and Karg 2013). They are capable of shifting their prey preferences in response to seasonal changes, weather conditions, habitat diversity, and nutritional needs (Orłowski and Karg 2013; Razeng and Watson 2015). The wide range of prey taxa consumed by aerial insectivores highlights their ecological role in ecosystems as well as their sensitivity to reductions in arthropod abundance. Aerial insectivores are particularly vulnerable to subtle ecological changes. They have experienced the steepest population declines of any group of North American birds, with the severity of these declines varying across the continent (Michel et al. 2016; Sauer et al. 2017). Therefore, studying the dietary preferences and niche overlap among aerial insectivores is crucial for understanding how ecologically and morphologically similar species can coexist within the same geographical area.

Aerial insectivorous birds, such as swallows, swifts, and swiftlets, forage on prey typically in open areas during daylight (Lourie and Tompkins 2000; Turner 2006). Due to their small size and agile foraging behaviour, it is challenging to observe and accurately detect their prey selection in the field (Symondson 2002). Furthermore, since they primarily prey on a wide range of small-bodied insects, identifying prey to a lower taxonomic level using conventional techniques, such as microscopic examination, is limited (Lourie and Tompkins 2000). Even when using molecular approaches, several factors can limit taxonomic resolution in dietary studies. For example, the rapid digestion in the bird's gut and difficulties in selecting or optimising primers often restrict the identification of prey to finer taxonomic levels (Chan et al. 2019; Mansor et al. 2022).

In recent years, the combination of improved primer sets, the capabilities of high-throughput sequencing, and advanced bioinformatics analysis has significantly enhanced dietary assessments of various vertebrates using metabarcoding of faecal DNA (Jedlicka et al. 2013; De Barba et al. 2014; Kartzinel et al. 2015). The application of these advanced molecular techniques has enabled non-invasive analysis of faecal samples to determine dietary preferences with

enhanced taxonomic resolution. Faecal DNA metabarcoding allows the identification of prey species that are challenging to detect through direct observation, especially in the case of aerial insectivores that forage for flying insects while airborne (Höhn et al. 2024). Such methods help to overcome the limitations of traditional molecular techniques by enabling taxonomic identification at a finer level, often down to the species.

In Malaysia, most dietary studies of insectivorous birds have primarily focused on forest environments (Moradi et al. 2009; Mansor et al. 2022; Shafie et al. 2023). Studies of these birds in forested landscapes provide valuable insights into their feeding behaviour and habitat requirements. However, research in mosaic landscapes, comprising urban, agricultural, and forest areas is equally important for a broader ecological understanding. Given the increasing fragmentation and land-use changes affecting tropical regions, understanding how these birds forage in such complex environments is essential for assessing their ecological roles and informing effective conservation strategies. Such studies can enhance the understanding of dietary patterns across different ecological contexts and help determine their roles as biological indicator species in agricultural landscapes.

Understanding niche segregation provides valuable insights into how resource use is partitioned and highlights the distinct ecological roles of aerial insectivorous species. This concept aligns with key ecological principles, such as niche theory, which explains patterns of biodiversity and ecosystem dynamics (Pianka 1974; Chase and Leibold 2009). By examining niche segregation, we address two fundamental questions: how do ecologically similar aerial insectivores coexist in shared habitats, and what unique ecological roles do generalist and specialist insectivores fulfil (Barnagaud et al. 2011).

Given the increasing urban presence of aerial insectivores such as swifts and swallows, particularly in town centers like Bentong, their large congregations may also raise concerns regarding sanitation, noise, and public health. Understanding their dietary ecology can help inform more sustainable urban wildlife management strategies, particularly in areas where nesting overlaps with human infrastructure. As such, this study not only contributes to avian ecological research but also has practical implications for mitigating human–wildlife conflict in rapidly urbanising regions.

Therefore, in this study, we analysed the diet of four aerial insectivorous bird species from two families: Apodidae, the House Swift (*Apus nipalensis*) and House-farmed Swiftlet (*Aerodramus* sp.); and Hirundinidae, the Barn Swallow (*Hirundo rustica*) and Pacific Swallow (*Hirundo tahitica*). This study aimed to: (1) identify the diversity of insect species consumed by co-occurring swallows and swifts in central Peninsular Malaysia; and (2) assess the degree of dietary overlap and segregation among these species. This study complements existing metabarcoding research by applying updated COI (ANML) primers and bioinformatics pipelines to investigate dietary partitioning among sympatric aerial insectivores in a mixed-use tropical landscape. The findings contribute to our understanding of how avian insectivores exploit diverse insect communities across human-modified habitats and potentially function as biological control agents, informing conservation and sustainable agroecosystem management.

Materials and methods

Study area

This study was conducted in the town area of Bentong, Pahang, in central Peninsular Malaysia (Fig. 1). This site was selected due to the high abundance of the aerial insectivorous species (Ismail et al. 2020; Mansor et al. 2020; Daud et al. 2022; Daud et al. 2024). Bentong is well documented as a roosting and nesting area of swallows and swifts, where these species predominantly roost on utility wires, building rooftops and shop lot signboards and under bridges in the town centre (Ismail et al. 2020; Mansor et al. 2020; Daud et al. 2022; Arazmi et al. 2025; Ismail et al. 2025a; Ismail et al. 2025b). The proliferation of swiftlet (*Aerodramus* sp.) house farms in the area allows for meaningful comparisons between these aerial insectivores. Bentong town centre provides a representative mosaic landscape where all four study species commonly coexist, making it an ideal setting to examine dietary patterns and potential niche partitioning in sympatric aerial insectivores.

To characterise the landscape profile of the study site, we used Google My Maps (https://www.google.com/maps/d/, accessed on 1 February 2022) to map land use within a 6 km radius from the swiftlet house farm. This radius was selected based on the average foraging range of swallows and swiftlets (Burhanuddin and Noor 2017; Daud et al. 2024; Arazmi et al. 2025). The resulting landscape area covered approximately 113.10 km^2 (11,310 hectares), comprising forest (71.70%), oil palm plantations (14.66%), urban development (11.73%), agricultural land with crops such as durian and coconut (1.08%), and water bodies (0.83%) (Fig. 2). This heterogeneous landscape offers a variety of habitat types that support the ecological requirements of the focal aerial insectivorous bird species.

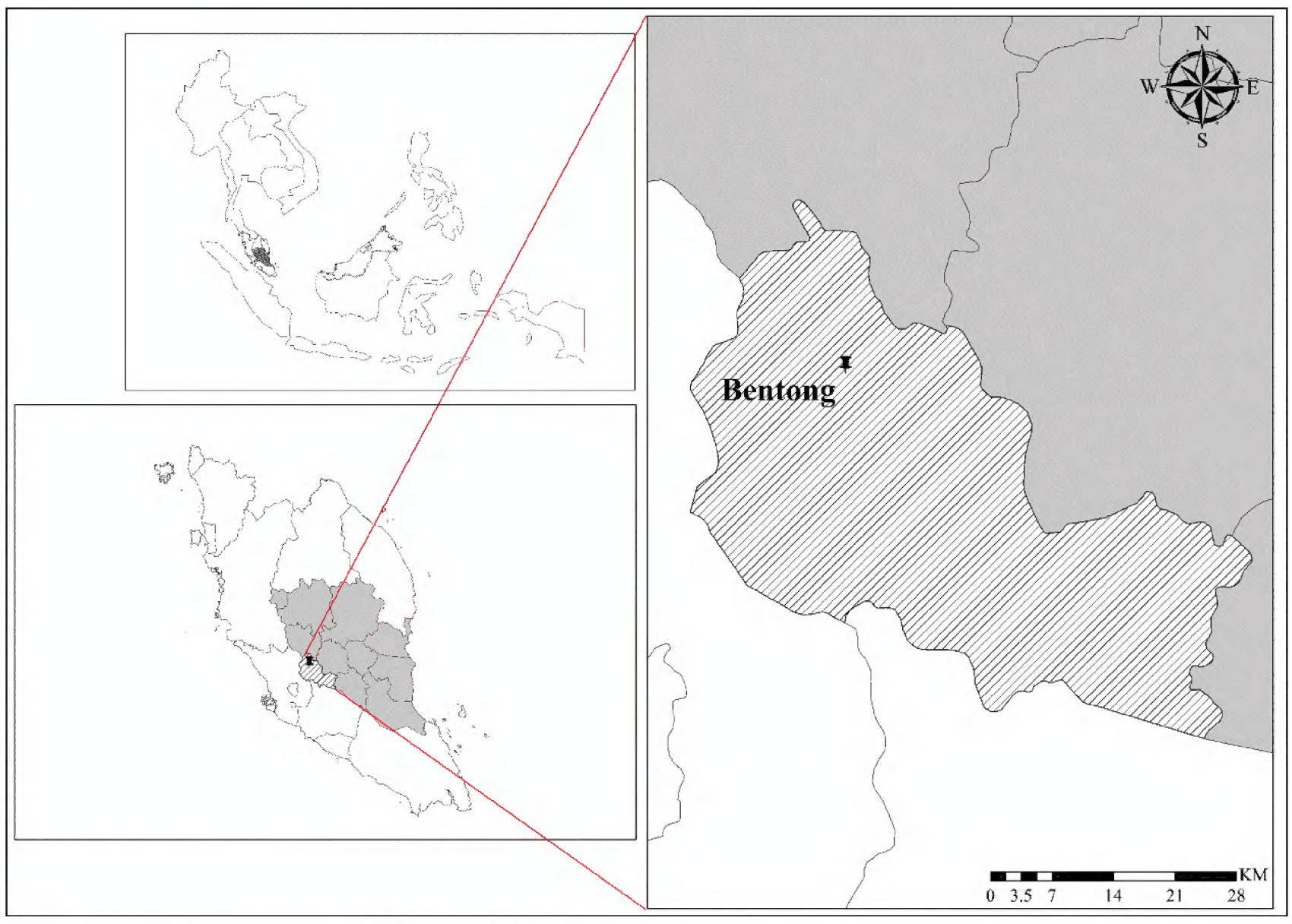

Figure 1. Study site in Bentong town, Pahang, for investigating four aerial insectivorous bird species.

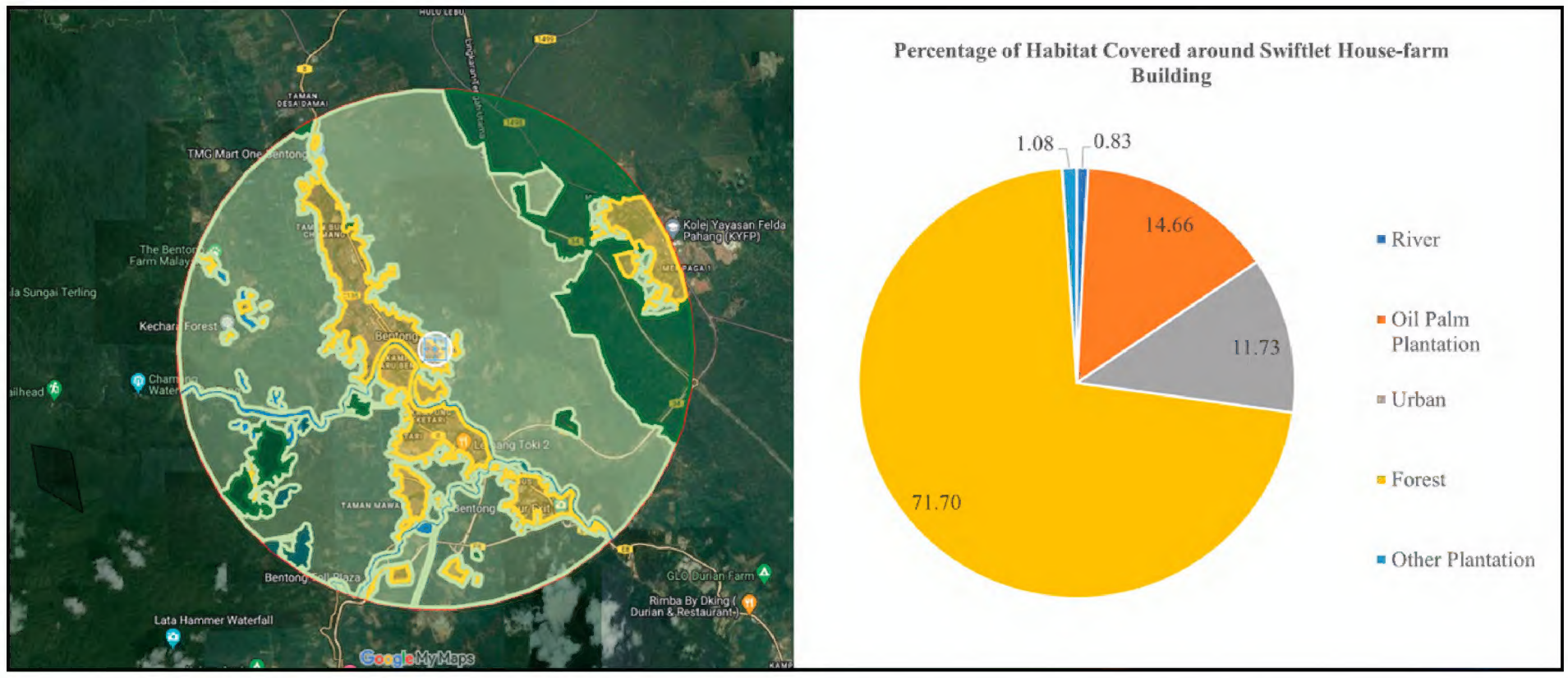

Figure 2. The landscape profile of habitat within a 6-km radius of the swiftlet house farm buildings in Bentong, Pahang.

Sample collection

For a fair comparison, faecal samples from all four species were collected on the same day in March 2022. Sampling was conducted daily over a one-week period to ensure consistent effort and enable for reliable dietary comparisons among species. Prior to sample collection, individuals were visually observed and identified using binoculars based on plumage characteristics and known nesting behaviours. This approach ensured that all faecal samples were reliably assigned to the correct species. Faecal samples of the House-farmed Swiftlet were collected directly from swiftlet farm buildings by placing plastic sheets (20 × 30 inches) beneath active nests. For the House Swift, Barn Swallow, and Pacific Swallow, samples were similarly collected using plastic sheets placed under their respective nesting or roosting sites around the town centre. Sampling for the House-farmed Swiftlet was conducted in the afternoon, as the birds typically return from foraging around midday (Daud et al. 2024). In contrast, samples from the other three species were collected at night, coinciding with their return to roost after dusk, given their diurnal foraging behaviour. To minimise contamination from human activity and traffic, plastic sheets were placed around midnight after shop closures, ensuring clean sample collection from confirmed roosting individuals. Faecal samples deposited on the plastic sheets were promptly collected with sterilised forceps to avoid cross contamination between species and preserved in 2 ml tubes with absolute ethanol. All samples were collected individually, with one dropping representing one individual from each of the aerial insectivorous species. During sampling, the faecal samples were stored in a cool box to maintain them at a cool temperature before they were stored in a freezer at −20 °C until DNA extraction.

DNA extraction and amplification

A total of 60 faecal samples were analysed, representing 15 individual samples for each of the four aerial insectivore species (House-farmed Swiftlet, House Swift, Barn Swallow, and Pacific Swallow). Arthropod DNA was extracted from the faecal samples collected from the four aerial insectivore species (using the

Qiagen, QIAmp DNA Stool Mini Kit, according to the manufacturer's instructions (QIAGEN, Hilden, Germany). DNA was eluted in 60 µl of elution buffer and was stored at −20 °C until further use. DNA concentration and purity were assessed using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, USA). To monitor contamination, negative controls were included in PCR steps. PCR amplification of a 180-bp fragment of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene was performed using the ANML primer set which is widely recognised for its effectiveness in DNA barcoding and arthropod species identification, with the forward primer LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer CO1-CFMRa (5'-GGWACTAATCAATTTCCAAATCC-3') (Jusino et al. 2017).

The DNA of the samples from each species was amplified using 25 µl of PCR mixture: 12.5 µl of NEXproTM e PCR 2× MasterMix solution, 8.5 µl of nuclease-free water, 1.0 µl each of forward and reverse primer (10 µM), and 2 µl of template DNA. The PCR reactions consisted of an initial denaturation step at 94 °C for 60 s, followed by the first amplification phase of five cycles at 94 °C for 60 s, 45 °C for 90 s, and 72 °C for 90 s. Then, the second amplification phase proceeded for 35 cycles at 94 °C for 60 s, 50 °C for 90 s, and 72 °C for 60 s and a final extension at 72 °C for 10 min (Hebert et al. 2003). The PCR products were examined using 2% agarose gel electrophoresis to detect the presence of DNA fragments. Electrophoresis was performed using 0.5X TAE buffer, consisting of Tris base, acetic acid and EDTA, to separate nucleic acids. Sequencing was performed using the Illumina MiSeq Sequencer (2× 250 bp; Ilumina, U.S.A.) according to standard protocols.

Bioinformatics and statistical analysis

To characterise dietary composition, assess interspecific differences, and evaluate trophic niche partitioning among four aerial insectivorous bird species, we applied detailed bioinformatic and statistical analyses. This combined framework enabled precise taxonomic identification of arthropod prey and supported comprehensive comparisons of diet profiles across species and taxonomic levels. The following section outlines the analytical pipeline used to process raw sequence data, assign taxonomic classifications, and evaluate dietary diversity and interspecific dietary overlap. The raw sequences in FASTQ format were processed using the QIIME2 platform, as detailed by Bolyen et al. (2019). The sequencing data, including both forward and reverse sequences, were imported into QIIME2 version 22.04.3. Before performing further analysis, the quality of raw sequences was evaluated using FastQC to confirm their suitability for subsequent steps.

The first step in the analysis was the demultiplexing of sequence reads, which was accomplished using the demux-paired function of the cutadapt plugin. This process identified the barcode sequences assigned during sequencing and accurately allocated the sequence reads to their respective samples based on the barcodes attached to the primers. The sequences then underwent a trimming process with the trim-paired function of fastp. This step was critical for removing the barcodes and primers attached to each sample's sequences and consequently, for refining the data and preparing them for the next stages of analysis. The merging of paired-end reads was performed next, using the fastp function to combine overlapping reads from paired-end sequences (both

forward and reverse sequences) into single-end reads. This step is particularly beneficial for downstream applications that rely on single-end reads, as it simplifies the data structure while retaining the necessary sequence information. After merging, the paired-end sequences were denoised and dereplicated, with chimeric sequences filtered out using the denoise-paired function in DADA2 (version 22.04.3). This process ensured that only high-quality, true sequences were retained, thus significantly improving the accuracy of the analysis. The next phase involved generating representative sequences, an abundance table, and a feature table using the feature-table plugin in QIIME2. The feature-table_summarize and feature-table_tabulate-seqs functions provided unique feature IDs for the representative sequences and clustered them into unique amplicon sequence variants (ASVs). Taxonomic assignment of the ASVs was then performed by querying the sequences against the NCBI GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences that showed a minimum of 98% similarity to the reference database were classified at the species level, those with 95% similarity at the genus level, and those with 90% similarity at the family level, according to the methods outlined by Mansor et al. (2020).

Using the taxa plugin in QIIME2, a diet dataset was constructed based on the taxonomic identifications derived from the feature-table plugin. This involved recording instances in individual samples and generating several metrics to summarise dietary preferences of the bird species. To characterise dietary composition, we used two complementary metrics: Relative Read Abundance (RRA) and Frequency of Occurrence (FOO) (Deagle et al. 2019). RRA represents the proportion of total sequencing reads assigned to each prey taxon, serving as an alternative metric to estimate the proportion of prey items in the diet of the aerial insectivores, reflecting the relative biomass consumed. FOO is defined as the percentage of samples in which a taxon was detected, reflecting how frequently it occurs in the diet across individuals. The FOO was calculated by dividing the number of detections of a particular prey item for a bird species by the total number of individuals of that bird species included in the study. These metrics help capture both the quantitative and qualitative aspects of prey consumption among species. The calculation of these metrics is elaborated in Suppl. material 1.

To visually represent the study results, seven visualisations were created using R (version 4.2.1) (Chambers 2008; R Development Core Team 2020). These included a heatmap to display the RRA of various prey taxa across multiple bird species, a bubble plot to visualise relationships between prey taxa, bird species, and their relative abundance. A network analysis was conducted using the bipartite package in R (Dormann et al. 2008) to visualise quantitative trophic interactions between bird species and their arthropod prey taxa. The input was a weighted interaction matrix based on Relative Read Abundance (RRA) values, where rows represented bird species and columns represented prey order. The resulting network highlights the strength and distribution of dietary links, allowing visual comparison of prey preference and overlap across species. Additionally, a chord diagram was generated using the circlize package (Finnegan et al. 2019) to represent proportional contributions of prey groups to each bird species' diet.

Finally, differences in diet composition between species were tested using permutational analysis of variance (PERMANOVA) of the distance matrix, with 'order, family, genus, species' and 'group' as predictor variables, with 9999

permutations. To identify which bird species significantly contributed to differences in diet selection based on order rank, a pairwise test with Bonferroni correction and 9999 permutations was performed. Diversity indices were calculated using the 'diversity' function in PAST software (version 4.03) (Hammer and Harper 2001) to measure arthropod diversity, dominance and evenness in bird diets. Dietary overlap and niche breadth were analysed using Pianka's Index (Ojk) and Levins' Index (B) in R software (version 4.2.1), with higher values indicating greater overlap and broader prey use, respectively. Pianka's Index was calculated using the spaa package (Zhang et al. 2016) to assess overlap in prey use between species, while Levins' Index was computed using the RInSp package (Zaccarelli et al. 2013) to evaluate dietary niche breadth.

Results

Sequencing results and taxon identification

DNA was successfully extracted and amplified from faecal samples of the four insectivorous bird species, yielding a total of 3,385,527 raw sequences. The number of sequences per individual sample ranged from 3,917 to 634,702, with a mean of 169,276.35 and a median of 95,802.50. After filtering, denoising, and merging, 2,494,044 sequences were retained, with sequence counts per sample ranging from 3,014 to 541,167 (mean ± standard deviation: 184.19 ± 12.23). From these sequences, 848 distinct ASVs were identified.

Of the 848 ASVs that passed quality control, taxonomic classification was successfully assigned across various levels, resulting in the identification of 13 orders, 96 families, 93 genera, and 65 species of prey in the diet of the four aerial insectivorous bird species. Taxonomic classification was performed based on sequence similarity thresholds (≥98% for species, ≥ 95% for genus, ≥ 90% for family), following the approach of Mansor et al. (2020). Many ASVs were identified only to genus or family level due to limited sequence resolution or lack of close matches in reference databases. This led to a situation where more ASVs are classified at the family or genus level than at the species level. The diets were predominantly composed of the Insecta, which was represented by 10 orders and 90 families, indicating a significant dominance of insects in these diets (Figs 3, 4). The remaining arthropods belonged to the class Arachnida, represented by three orders and six families.

Relative read abundance and frequency of occurrence

The results emphasise the dietary diversity of these aerial insectivorous birds. While certain arthropod orders were commonly consumed across all species, others appeared less abundant or were selectively exploited by specific bird species, indicating dietary specialisation. The Hymenoptera, Diptera, and Coleoptera were the most common prey orders in the diet of the four bird species. The proportion of prey from the order Hymenoptera was highest in the diets of the Barn Swallow and House-farmed Swiftlet, accounting for 38.6% and 36.2% of their respective diets (Figs 3, 4). In contrast, the Pacific Swallow and House Swift predominantly consumed insects from the order Diptera, which comprised 63.8% and 67.3% of their

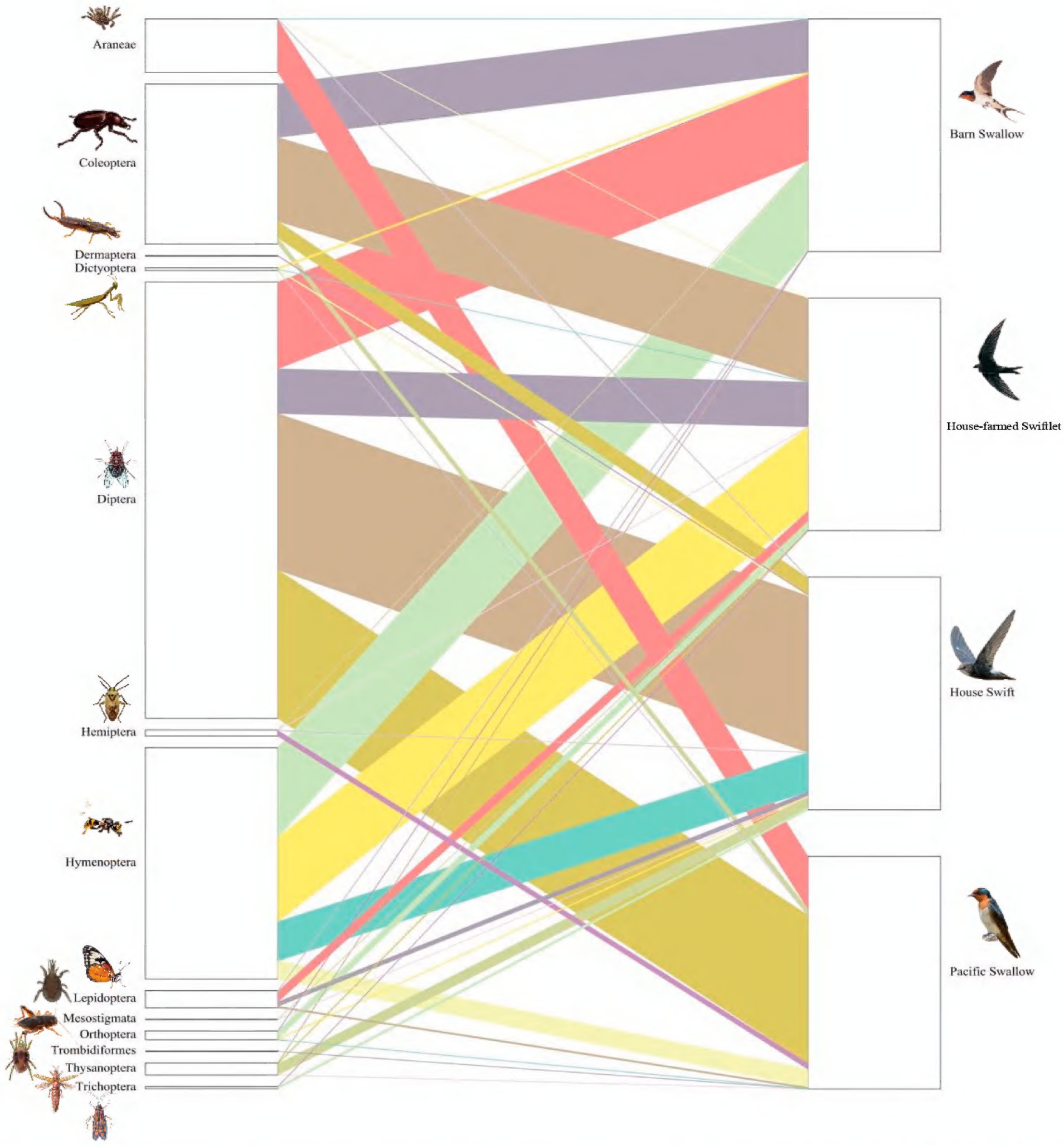

Figure 3. Bipartite diagram showing the relative read abundance (RRA) of arthropod orders in the diets of four sympatric aerial insectivorous bird species in central Peninsular Malaysia. Wider bars indicate a higher proportion of sequencing reads assigned to that prey taxon, reflecting its greater dietary contribution.

diets, respectively. Coleoptera also contributed substantially to the diets of the Barn Swallow and House-farmed Swiftlet, at 23.0% and 36.1% respectively, although it was much less prevalent in the diets of the House Swift (7.1%) and Pacific Swallow (2.7%). These patterns indicate varying degrees of reliance on the three dominant insect orders among bird species (Figs 3, 4). The FOO of Hymenoptera, Diptera, and Coleoptera was 100% across all sampled individuals of the four bird species, indicating that these three orders were consistently present in every faecal sample analysed (Fig. 5).

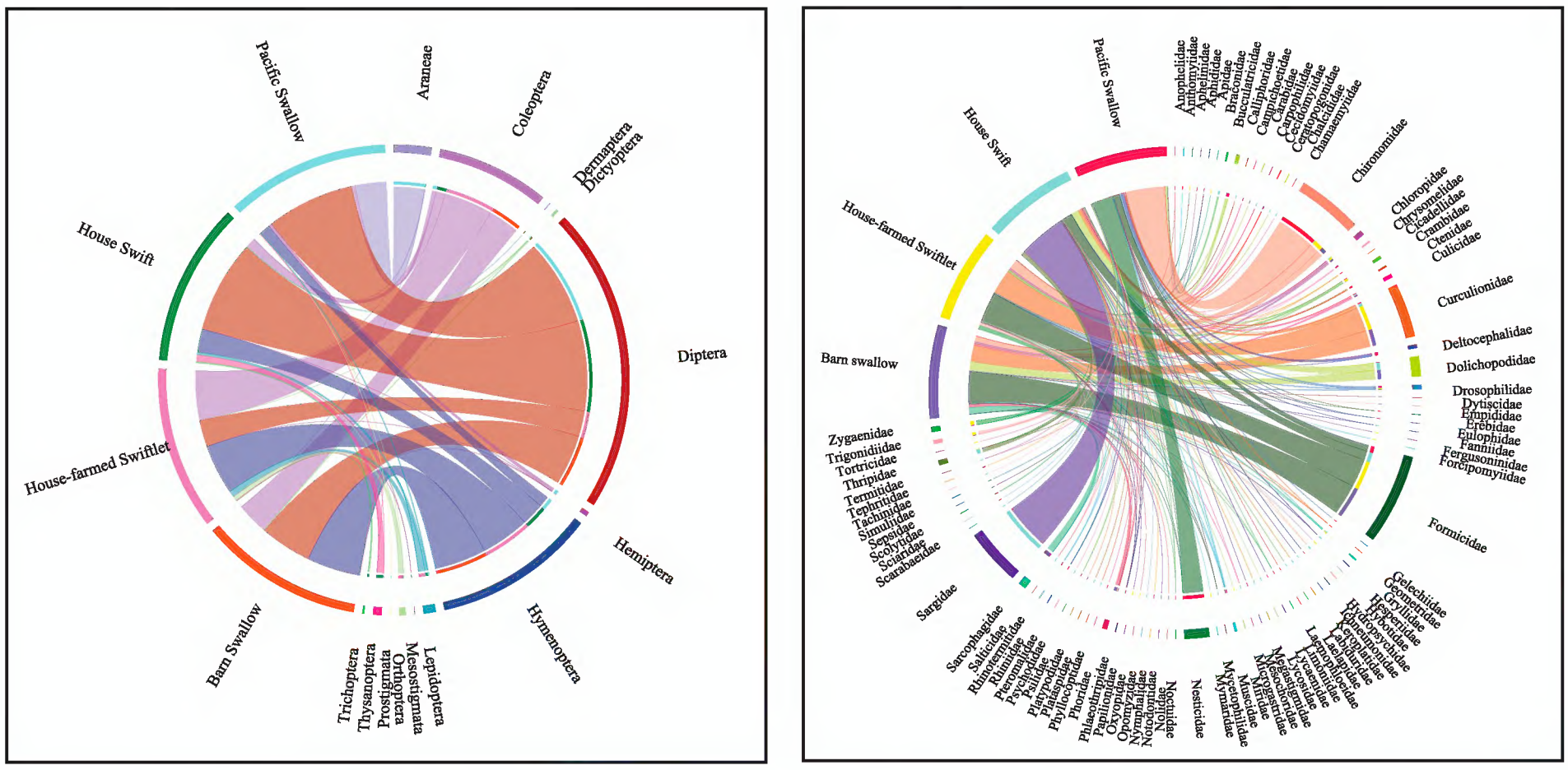

Figure 4. Chord diagrams illustrating dietary composition of four aerial insectivorous bird species in central Peninsular Malaysia based on relative read abundance (RRA) of arthropod prey: a) Prey composition at the order level; b) Prey composition at the family level.

In addition to these dominant orders, other orders such as the Blattodea, Lepidoptera, Hemiptera, Thysanoptera, Orthoptera (Insecta), and Araneae (Arachnida) were also present in the diets of all four bird species, although at varying levels of RRA (Fig. 3). Among the most abundant prey taxa detected in the diets of the four aerial insectivore species, the families Formicidae, Curculionidae, Dolichopodidae, Chironomidae, Stratiomyidae, and Nesticidae were particularly notable, each comprising more than 10% of the total RRA across all samples. In addition, other classes were identified in the samples, although they comprised a lower percentage of the total readings in most instances; the class Arachnida was represented by three orders, the Araneae, Mesostigmata, and Trombidiformes and included six families, the Ctenidae, Laelapidae, Lycosidae, Nesticidae, Oxyopidae, and Phyllocoptidae (Fig. 4).

Dietary overlap and segregation

In the evaluation of the prey consumption patterns of the four insectivorous species, the aggregation of insect read abundance data at the order level revealed significant niche overlap between the bird species. The results of the PERMANOVA showed that dietary composition at a higher taxonomic level did not differ significantly between the bird species ($F = 0.9459$, $R^2 = 6.07$, $p = 0.5092$). The Bonferroni-corrected p-values were equal to one and in the case of the comparison between the House-farmed Swiftlet and Pacific Swallow, the p-value was less than one but still above the significance threshold (Bonferroni-correction: $F = 1.558$, $p = 0.5676$), suggesting that there was no significant difference in order rank between these species. Besides, the mean degree of overlap was identified as less than 0.4% Ojk (range between ≤ 0.01 to ≥ 0.4), where values near 0 indicate minimal overlap between all the four species (Table 1).

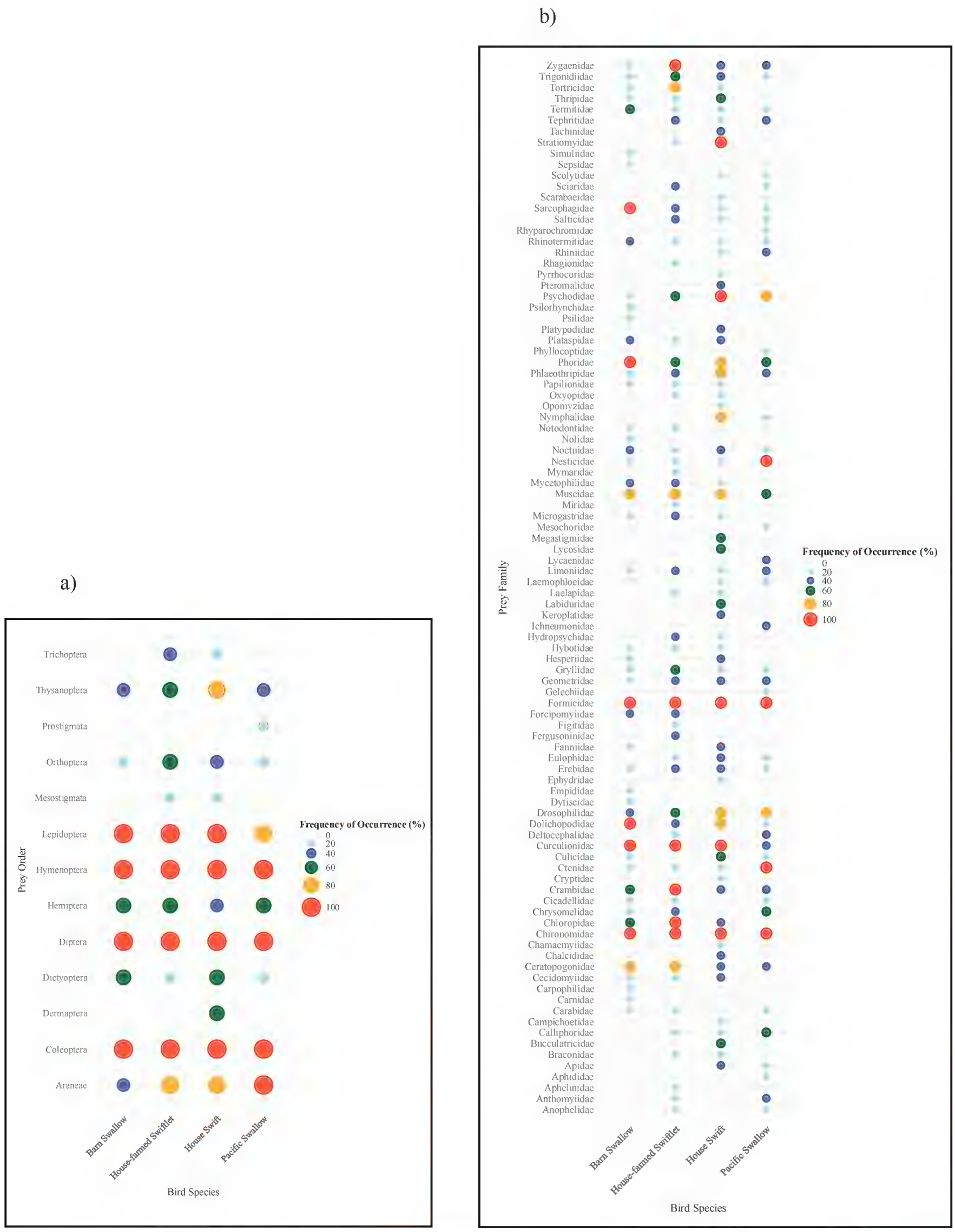

Figure 5. a. Frequency of occurrence (FOO) of arthropod prey orders in faecal samples from four aerial insectivorous bird species in central Peninsular Malaysia; **b.** The frequency of occurrence (FOO) of arthropod prey families in faecal samples from four aerial insectivorous bird species in central Peninsular Malaysia.

Although there was dietary overlap between the four insectivorous species at the higher taxonomic level, the proportions of different insect orders consumed varied. Further analysis revealed that certain insect orders were detected only in specific bird species. For example, Dermaptera was

Table 1. Pianka overlap index (Ojk) calculated at the order level to assess dietary overlap among four species of aerial insectivorous birds in central Peninsular Malaysia.

Aerial insectivorous birds	Barn Swallow	House-farmed Swiftlet	House Swift	Pacific Swallow
Barn Swallow	–	0.215	0.158	0.055
House-farmed Swiftlet	–	–	0.253	0.069
House Swift	–	–	–	0.041

found exclusively in the House Swift (0.22%), while the Trichoptera was detected in both the House Swift and the House-farmed Swiftlet. In addition, the arachnid order Mesostigmata was detected in the faeces of the House Swift and House-farmed Swiftlet, although at comparatively low RRA levels of 0.01% and 0.002%, respectively. The arachnid order Trombidiformes was detected solely in the Pacific Swallow (Suppl. material 1: table S1).

At a finer taxonomic scale (family, genus, and species), dietary preferences differed significantly between the bird species. At the family level, prey composition showed significant variation (PERMANOVA: $F = 1.813$, $R^2 = 7.634$, $p = 0.0033$). This pattern became even more distinct at the genus ($F = 2.038$, $R^2 = 8.656$, $p = 0.0002$) and species levels ($F = 2.378$, $R^2 = 8.670$, $p = 0.0001$), indicating increased dietary differentiation with lower taxonomic resolution. The Barn Swallow exhibited the highest Shannon diversity index ($H' = 1.836$) at the prey family level, indicating a diverse diet with a balanced consumption of various prey families ($E = 0.1255$). The dominant families in the Barn Swallow's diet included the Formicidae (40.26%), Curculionidae (21.33%), Dolichopodidae (11.62%), Sarcophagidae (7.94%), and Chironomidae (7.13%) (Fig. 4). In contrast, the House Swift showed the lowest diversity ($H' = 1.564$), suggesting a less diverse diet at family rank, indicating a broad range of prey families, with Stratiomyidae being the most preferred (60.27%) (Fig. 4).

Overall, 65 prey species were identified in the diets of the four aerial insectivore species. Notable prey species included *Polyrhachis lepida* (31.49% in Barn Swallow and 48.27% in House-farmed Swiftlet diets), *Chrysosoma vittatum* (19.88% in the Barn Swallow diet), *Polyrhachis danum* (16.09% in House-farmed Swiftlet and 10.50% in Barn Swallow diets), *Polyrhachis* sp. 1 (16.09% in House-farmed Swiftlet and 10.50% in Barn Swallow diets), *Chironomus* sp. 1 (48.25% in the Pacific Swallow diet), *Chrysomya chani* (11.01% in the Pacific Swallow diet), *Hishimonus phycitis* (18.13% in the Pacific Swallow diet), *Metioche vittaticollis* (9.69% in the House-farmed Swiftlet diet), and *Microchrysa flaviventris* (86.81% in the House Swift diet) (Fig. 6 and Suppl. material 1: table S1).

In summary, the Barn Swallow showed the highest dietary diversity ($H' = 2.11$) and evenness ($E = 0.3438$), indicating a varied and balanced diet at the species level. In contrast, the House Swift exhibited the lowest diversity ($H' = 0.68$) among the four species but consumed the widest range of arthropod species. The Levins' dietary niche breadth index demonstrates clear variation among the studied species, highlighting differences in their ecological roles and resource utilization strategies. Barn Swallow registered the highest niche breadth index ($B = 5.79$), while House swift showed the lowest niche breadth ($B = 1.32$), reflecting differences in species' adaptability within similar environmental conditions (Fig. 7).

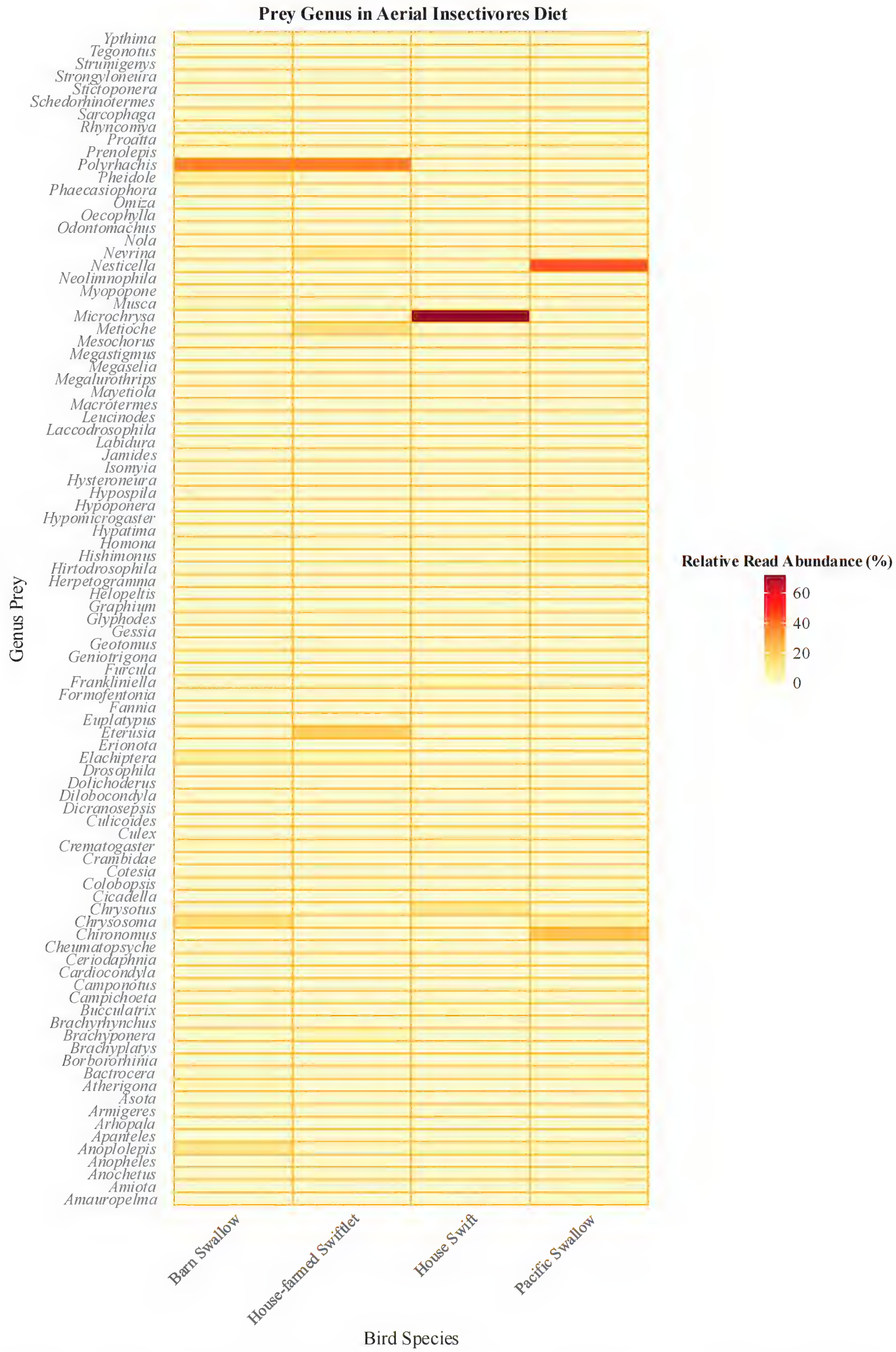

Figure 6. Heatmap of the relative read abundance of arthropod prey genera in faecal samples from four aerial insectivorous bird species in central Peninsular Malaysia.

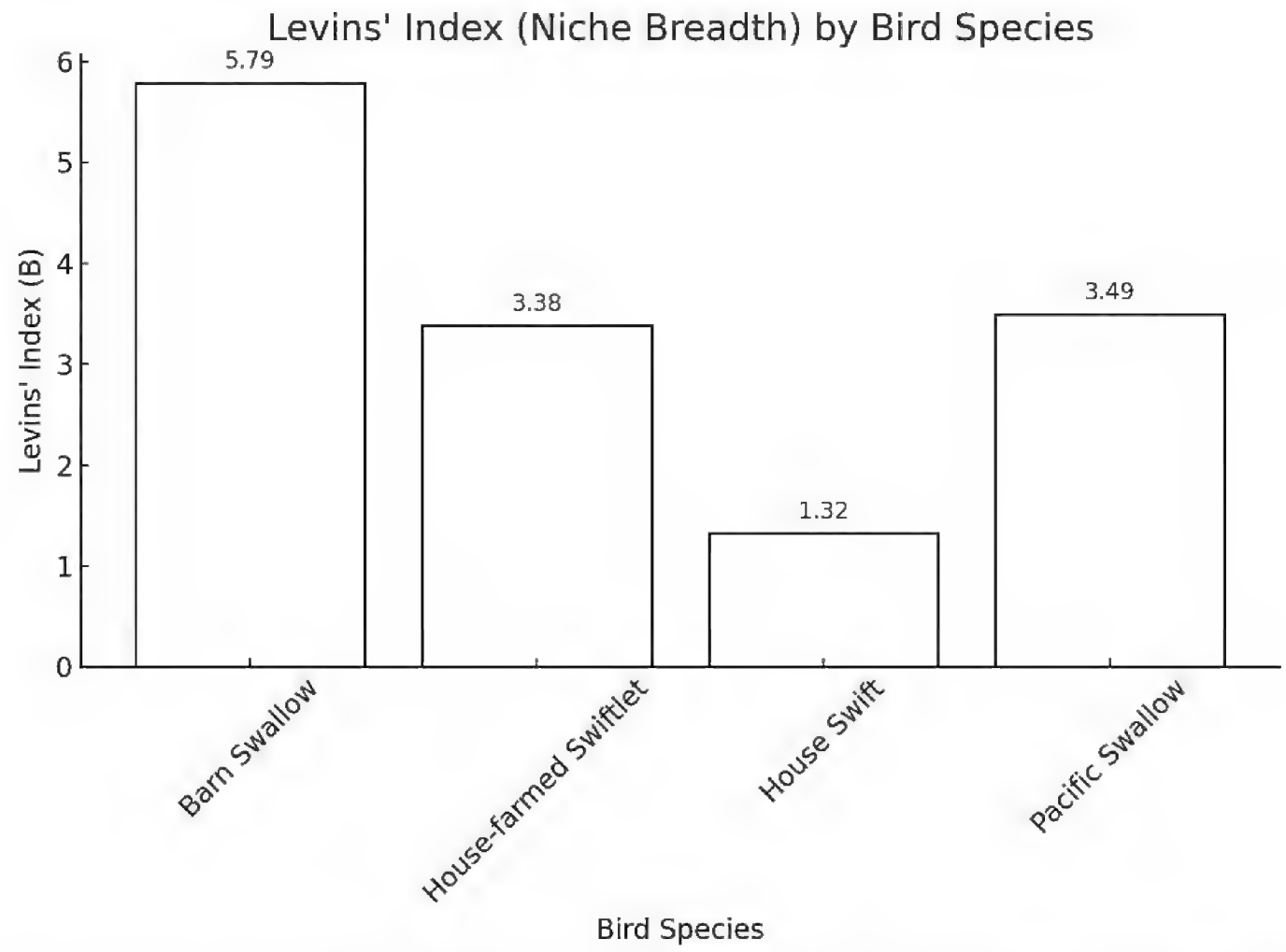

Figure 7. Levins' Index (B) representing dietary niche breadth between four different species of aerial insectivorous birds in Central Peninsular Malaysia.

Discussion

Dietary profile

Our study provides novel molecular dietary data that expand the understanding of the dietary ecology of four aerial insectivorous bird species in central Peninsular Malaysia. The results of our metabarcoding analysis indicate that the ANML primer pair is a reliable tool for generating high-quality DNA sequence data for insects and related taxa in tropical regions. This approach offers significant advantages over conventional morphological methods, which often limit prey identification to higher taxonomic levels (e.g., order or family) and result in many prey items remaining unidentified due to digestion or lack of distinguishing morphological features. For example, Lourie and Tompkins (2000) found that insect remains in Malaysian swiftlet diets were often only identified to the order level, such as 'Diptera' or 'beetle fragments. In contrast, the analysis of the RRA of arthropod prey taxa in each sample demonstrated that the metabarcoding data effectively captured a diverse array of arthropod prey in the diets of these birds. We analysed a total of 848 ASVs from faecal samples and identified 65 arthropod species, predominantly insects. The prey items could be divided into three primary categories, terrestrial invertebrates, aerial invertebrates, and aquatic invertebrates, predominantly from the orders Hymenoptera, Coleoptera, and Diptera. This pattern at the order level (a higher taxonomic classification) is consistent with findings from a previous dietary analysis of *Apus nipalensis* (Chan et al. 2019) and the diet profiling of *Aerodramus* sp. and *Hirundo rustica* (Mansor et al. 2020).

In this study, we used a multi-level taxonomic approach from order to species to examine dietary variation and overlap among four aerial insectivorous bird species. This method allowed us to explore how patterns of trophic segregation change across different levels of taxonomic resolution. The detailed taxonomic breakdown provides valuable insights into the dietary preferences

and ecological interactions of these bird species. At the order level, dietary composition was largely similar among species, as shown by the non-significant PERMANOVA results ($p = 0.5092$). However, clear differences in diet emerged at finer taxonomic levels (family, genus, species), emphasising the importance of detailed classification for detecting ecological differences. By analysing data across multiple levels, we were able to identify where dietary divergence becomes most apparent and gain a better understanding of how these species partition food resources (Clare 2014). This underscores the usefulness of multi-level dietary analysis in trophic ecology, especially for studying the ecological roles of species that coexist in the same environment.

Dietary overlap at the order level

The dominance of these insect orders, each with an FOO of 1.0 for the bird species studied, highlights the dietary overlap at the order level. The dietary similarities between these bird species are likely due to the close proximity of their nesting and roosting sites, resulting in the shared exploitation of abundant insect prey from the same orders within the surrounding habitat. The dominance of these insect orders is likely driven by the surrounding forest structure and vegetation complexity, which support high arthropod diversity. Landscape analysis within a six-kilometer radius of the town centre showed that 87.38% of the area is forested, indicating that the mosaic landscape that is rich in vegetation and influenced by abiotic factors plays a key role in sustaining diverse and accessible arthropod prey for aerial insectivores. This highlights the importance of retaining forested areas or green spaces within urban environments, as these habitats support insect populations that are vital food sources for aerial insectivorous birds.

The aerial foraging behaviour and parental investment strategies of these birds (Daud et al. 2022) likely contribute to the observed dietary overlap. Breeding individuals may optimise energy use by adjusting foraging frequency and proximity to nesting sites in response to prey abundance (Bloch et al. 2024; Daud et al. 2024). During periods of high insect abundance, many birds optimise energy use by foraging closer to their nests and consuming high-quality, diverse food, which increases the survival rate of their young (Winkler et al. 2013). The four bird species studied likely consume similar insect orders due to their behavioural plasticity which allows them to adjust their foraging strategies in response to fluctuating prey availability, including by targeting a diverse array of insect prey. Such diversity in prey species likely contributes to the minimisation of competition despite the high dietary overlap between these predators. As long as food resources remain abundant, interspecific competition is kept low. However, if prey availability declines, the degree of dietary overlap may intensify, potentially leading to increased competition (Macarthur and Levins 1967).

Dietary segregation at family, genus and species level

At a finer taxonomic scale of prey family, genus, and species dietary preferences differed significantly between the four bird species (Fig. 7). The diversity and distinct types of prey of these bird species suggests dietary segregation within the urban forest ecosystem. Of the arthropod families, the Formicidae was the most dominant in the diets of the bird species studied, but with varying

proportions of genera and species. The dominance of the Formicidae in the diets is likely due to their abundance in both urban and forested ecosystems, where they constitute a substantial portion of animal biomass (Griffiths et al. 2018) and play a crucial role as keystone species and provide an essential food source for insectivores (Parker and Kronauer 2021).

The diet of the Barn Swallow consisted of 50 families, 43 genera, and 24 insect species and was heavily dominated by the Formicidae, which included 14 species of alate and non-alate ants. Of these, *Polyrhachis lepida* was the most abundant prey species, making up approximately 31.49% of the total insect mass consumed. The analysis of the diet of the Pacific Swallow identified 47 families, 30 genera and 22 arthropod species, with *Chironomus* sp. 1, *Hishimonus phycitis* and *Chrysomya chani* standing out as the most prominent in their diet. The Diptera families, Chironomidae, Calliphoridae, and Drosophilidae, were dominant in the diet of the Pacific Swallow followed by the Araneae, including the Nesticidae and Ctenidae. The diet of the House-farmed Swiftlet comprised 56 families, 40 genera and 26 species of arthropod prey with varying levels of abundance, with *Polyrhachis lepida*, *Polyrhachis danum*, and *Polyrhachis* sp. being the most dominant species. These results indicate that the genus *Polyrhachis* and the family Formicidae were the most dominant taxonomic groups in the diet of House-farmed Swiftlet. In contrast, the House Swift's diet showed a markedly different profile, consisting of 12 orders, 50 families, 33 genera and 34 species. The Stratiomyidae and Formicidae were the most dominant families in the diet of the House Swift, while the most abundant species was *Microchrysa flaviventris*.

The dietary profiles revealed a broad range of arthropod prey consumed by these aerial insectivores. While some prey families were commonly consumed across all four species, others were infrequent or appeared to be selectively targeted by only certain bird species, indicating a degree of dietary specialisation. The differences in the RRA between the insectivore species demonstrate the complex nature of their feeding ecology and other ecological factors, such as habitat and prey availability, which may influence prey selection.

Ecological drivers to dietary preference

Variation in foraging altitude and habitat use likely explains the differences in dietary preferences between these species. For instance, the foraging altitude of aerial insectivores such as the Purple Martin (*Progne subis*) is correlated with the body size of ant prey, with lighter insects more likely to ascend to higher altitudes (Helms et al. 2016). On the other hand, the Barn Swallow forages at an average maximum altitude of 167 m (Dreelin et al. 2018), while the House-farmed Swiftlet typically forages between 80 m and 100 m (Fujita and Leh 2020). These differences in foraging altitude may contribute to dietary segregation between species, thereby reducing interspecific competition (Mansor et al. 2022).

Optimal foraging theory suggests that animals engage in behaviours that maximise their net energy intake per unit of time (Pyke et al. 1977). Given their flexibility, aerial insectivorous birds prioritise prey that offers the highest energetic efficiency which is influenced by prey density, foraging altitude, and ease of capture (Roslin et al. 2017). Central place foraging theory posits that foraging closer to nesting sites is more energy-efficient, resulting in variation in diet composition based on prey availability and foraging distance (Charnov

1976; Bell 1990). These factors may contribute to the dietary preferences observed in this study, with each bird species adapting its foraging strategy to balance energy expenditure and prey acquisition through the consumption of a diverse array of prey species (Whelan 2001).

Our sampling of Barn Swallows took place in March, when they typically consume more food and a high diversity of prey species in preparation for their return to breeding grounds (Ismail et al. 2025a). Generalist feeders such as the Barn Swallow consume a more diverse diet which allows them to exploit a broader range of prey resources in response to environmental changes. This is likely influenced by its migratory behaviour, which requires adaptability to varying habitats and prey availability (Ismail et al. 2025a). This is supported by our analysis, where the Barn Swallow exhibited the highest dietary diversity and the widest niche breadth, indicating a generalist foraging strategy.

Species specialisation in dietary preference was detected in House Swift, which showed the lowest Shannon Diversity Index and the narrowest niche breadth, indicating a specialist feeding strategy focused on a limited range of prey types. Being resident species with large populations, swifts can adjust their foraging strategy by focusing on specific arthropod groups, consistent with optimal foraging theory, thereby minimising the costs associated with foraging, such as time and energy expenditure. The understanding of these dynamics is crucial for grasping the ecological interactions that shape the dietary strategies of aerial insectivorous birds in tropical ecosystems.

The findings offer important conservation insights for aerial insectivorous birds in increasingly fragmented and urbanised landscapes. Dietary segregation at finer taxonomic scales reflects resource partitioning, which may help minimise competition among coexisting species. Conserving arthropod diversity, particularly within forest remnants and green corridors is vital for supporting insectivore diets and maintaining ecosystem services such as natural pest regulation. Furthermore, DNA-based dietary tools provide a non-invasive means to track changes in prey availability, habitat quality, and urban ecological conditions. As cities continue to expand, protecting insect-rich habitats and incorporating biodiversity considerations into urban planning will be essential for sustaining aerial insectivore populations and overall ecological resilience.

Conclusions

Our comprehensive investigation of the diets of four aerial insectivorous bird species in Bentong town, Pahang, provides valuable insights into their feeding behaviours and food preferences. The use of the ANML primer pair in dietary metabarcoding proved to be effective for generating high-quality DNA sequences which facilitated the precise identification of a diverse range of arthropod prey. This approach proved instrumental in uncovering 13 orders, 96 families, 93 genera, and 65 species in the diets of these birds in central Peninsular Malaysia. The ANML primer's broad taxonomic coverage and reliability highlight its potential as a powerful tool for dietary studies in ecological research.

Hymenoptera, Diptera, and Coleoptera were the most common insect orders in the diets of all four bird species, highlighting their ecological importance as prey. Differences in dietary preferences suggest these birds partition their diets, allowing them to coexist in the same habitat. Barn Swallows (Hirundinidae)

act as generalist feeders, consuming a wide range of prey, while House Swifts (Apodidae) are more specialised. Despite some overlap in their diets, their feeding habits reflect adaptations to local prey availability and efficient foraging to support parental care. Generalist species, like Barn Swallows, enhance ecosystem resilience by adapting to changing environments, while specialist species, like House Swifts, play key roles in specific ecological interactions but are more sensitive to habitat or prey changes.

While the study was limited to a single locality and season, the patterns observed offer important baseline data for understanding aerial insectivore ecology in tropical urbanised landscapes. Broader studies across multiple regions and temporal scales are encouraged to evaluate the consistency of these patterns throughout Peninsular Malaysia and Southeast Asia.

The present findings have important practical implications for conservation and sustainable agriculture. Preserving habitats that support both generalist and specialist aerial insectivores is essential for maintaining biodiversity and ecological balance, particularly in increasingly fragmented landscapes. Insights into the dietary composition and niche differentiation of these birds can inform habitat management strategies tailored to species with different ecological roles. Moreover, understanding their feeding habits provides a scientific basis for integrated pest management, where both generalist and specialist species contribute to natural pest regulation, potentially reducing the need for chemical pesticide use in agriculture. Recognising the distinct functional roles of these insectivores highlights the importance of targeted conservation planning and biodiversity-friendly land-use practices that support ecosystem resilience.

Acknowledgments

We thank the owner of the swiftlet house-farm and all shop lot owners in the town area of Bentong for their friendly support for sample collection by permitting us to carry out work on their properties.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study was funded by the Fundamental Research Grant Scheme (FRGS), Ministry of Higher Education Malaysia, under grant FRGS/1/2020/STG03/UKM/02/5.

Author contributions

FNA, NAI and UNSD retrieved the faecal samples collection. FNA extracted and amplify the DNA, and running the bioinformatics analysis. MSM conceptualised the study design. FNA and NAI analysed and interpreted the data using R and Past software's. FNA and MSM led the manuscript writing. The authors read and approved the final manuscript.

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Data availability

All data needed in this article are present in the Dryad Digital Repository (https://doi.org/10.5061/dryad.3ffbg79tx).

References

Arazmi FN, Ismail NA, Daud UNS, Mansor MS (2025) DNA Metabarcoding Unveils Habitat-Linked Dietary Variation in Aerial Insectivorous Birds. Animals (Basel) 15: 974. https://doi.org/10.3390/ani15070974

Barnagaud JY, Devictor V, Jiguet F, Archaux F (2011) When species become generalists: On-going large-scale changes in bird habitat specialization. Global Ecology and Biogeography 20: 630–640. https://doi.org/10.1111/j.1466-8238.2010.00629.x

Bell WJ (1990) Central place foraging. In: Bell WJ (Ed.) Searching Behaviour: The behavioural ecology of finding resources. Springer Netherlands, Dordrecht, 171–187. https://doi.org/10.1007/978-94-011-3098-1_12

Beskardes V (2020) Habitat Selection Of Insectivorous Birds In Western Black Sea Region Of Turkey. Applied Ecology and Environmental Research 18(6): 7551–7562. https://doi.org/10.15666/aeer/1806_75517562

Bloch I, Troupin D, Toledo S, Nathan R, Sapir N (2024) Combining radio-telemetry and radar measurements to test optimal foraging in an aerial insectivore bird. bioRxiv: 2024: 580922. https://doi.org/10.7554/eLife.96573.2

Bolyen E, Rideout JR, Dillon M, Bokulich N, Abnet C, Al-Ghalith G, Alexander H, Alm E, Arumugam M, Asnicar F, Bai Y, Bisanz J, Bittinger K, Brejnrod A, Brislawn C, Brown CT, Callahan B, Caraballo Rodríguez A, Chase J, Caporaso J (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37: 1. https://doi.org/10.1038/s41587-019-0209-9

Burhanuddin M, Noor HM (2017) Ranging behaviour of edible nest swiftlet (*Aerodramus* sp.) in Kuala Langat district, Selangor, Malaysia. Malaysian Applied Biology 46: 59–66.

Chambers JM (2008) Software for data analysis: programming with R. Vol. 2, Springer.

Chan KS, Tan J, Goh WL, Cranbrook Eo (2019) Diet profiling of house-farm swiftlets (Aves, Apodidae, *Aerodramus* sp.) in three landscapes in Perak, Malaysia, using high-throughput sequencing. Tropical Ecology 60: 379–388. https://doi.org/10.1007/s42965-019-00040-1

Charnov EL (1976) Optimal foraging, the marginal value theorem. Theoretical Population Biology 9: 129–136. https://doi.org/10.1016/0040-5809(76)90040-X

Chase JM, Leibold MA (2009) Ecological niches: linking classical and contemporary approaches. University of Chicago Press.

Clare EL (2014) Molecular detection of trophic interactions: Emerging trends, distinct advantages, significant considerations and conservation applications. Evolutionary Applications 7: 1144–1157. https://doi.org/10.1111/eva.12225

Daud UNS, Ismail N, Nor S, Mansor MS (2022) Biparental incubation behavior in the domestic house-farmed swiftlets (*Aerodramus* sp.) in central Peninsular Malaysia. Animal Biology (Leiden, Netherlands) 72: 1–11. https://doi.org/10.1163/15707563-bja10066

Daud UNS, Ismail NA, Ramasamy K, Arazmi NFN, Nor SM, Mansor MS (2024) Using closed-circuit infrared camera to assess biparental provisioning behaviour and foraging pattern of house-farmed swiftlets (*Aerodramus* sp.). Animal Biology (Leiden, Netherlands) 1: 1–13. https://doi.org/10.1163/15707563-bja10138

De Barba M, Miquel C, Boyer F, Mercier C, Rioux D, Coissac E, Taberlet P (2014) DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: Application to omnivorous diet. Molecular Ecology Resources 14: 306–323. https://doi.org/10.1111/1755-0998.12188

Deagle BE, Thomas AC, McInnes JC, Clarke LJ, Vesterinen EJ, Clare EL, Kartzinel TR, Eveson JP (2019) Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? Molecular Ecology 28: 391–406. https://doi.org/10.1111/mec.14734

Dormann CF, Gruber B, Fründ J (2008) Introducing the bipartite package: analysing ecological networks. interaction 1: 8–11.

Dreelin RA, Shipley JR, Winkler DW (2018) Flight behavior of individual aerial insectivores revealed by novel altitudinal dataloggers. Frontiers in Ecology and Evolution 6: 182. https://doi.org/10.3389/fevo.2018.00182

Dunoyer J, Lalla K, Fraser K, Elliott K (2024) Diet is associated with age and habitat, not foraging range, in purple martins: Implications for aerial insectivores as indicators. Canadian Journal of Zoology 102: 7. https://doi.org/10.1139/cjz-2023-0164

Finnegan A, Sao SS, Huchko MJ (2019) Using a chord diagram to visualize dynamics in contraceptive use: Bringing data into practice. Global Health, Science and Practice 7: 598–605. https://doi.org/10.9745/GHSP-D-19-00205

Fujita M, Leh C (2020) The feeding ecology of edible-nest swiftlets in a modified landscape in Sarawak. Anthropogenic Tropical Forests: Human–Nature Interfaces on the Plantation Frontier, 401–415. https://doi.org/10.1007/978-981-13-7513-2_19

Griffiths HM, Ashton LA, Walker AE, Hasan F, Evans TA, Eggleton P, Parr CL (2018) Ants are the major agents of resource removal from tropical rainforests. Journal of Animal Ecology 87: 293–300. https://doi.org/10.1111/1365-2656.12728

Hammer Ø, Harper DA (2001) Past: Paleontological statistics software package for educaton and data anlysis. Palaeontologia Electronica 4: 1.

Hebert P, Cywinska A, Ball SL, Dewaard J (2003) Biological identification through DNA barcodes. Proceedings. Biological Sciences 270: 313–321. https://doi.org/10.1098/rspb.2002.2218

Helms JA, Godfrey AP, Ames T, Bridge ES (2016) Predator foraging altitudes reveal the structure of aerial insect communities. Scientific Reports 6: 28670. https://doi.org/10.1038/srep28670

Höhn D, Masello JF, Kümmel MN, Griep S, Goesmann A, Quillfeldt P (2024) Nestling Diet of Two Sympatric Insectivorous Passerines in Different Habitats—A Metabarcoding Study. Birds 5: 67–89. https://doi.org/10.3390/birds5010005

Ismail N, Jufri A, Daud UNS, Nor S, Mansor MS (2020) Short Communication: Roosting behavior of Wintering Barn Swallow (*Hirundo rustica*) in Peninsular Malaysia. Biodiversitas (Surakarta) 21(2): 661–665. https://doi.org/10.13057/biodiv/d210231

Ismail NA, Daud UNS, Arazmi NFN, Nor SM, Mansor MS (2025a) Dietary shifts in Barn Swallow *Hirundo rustica* passage and wintering in Peninsular Malaysia: From the early to late migratory season. European Journal of Wildlife Research 71: 1–12. https://doi.org/10.1007/s10344-025-01904-9

Ismail NA, Syafiqah Daud UN, Najihah Arazmi NF, Md Ali NB, Nor SM, Mansor MS (2025b) Morphological Sex Determination of East Asian Barn Swallows (*Hirundo rustica*) in

Tropical Wintering Region. Pertanika Journal of Tropical Agricultural Science 48. https://doi.org/10.47836/pjtas.48.1.02

Jedlicka JA, Sharma AM, Almeida RPP (2013) Molecular tools reveal diets of insectivorous birds from predator fecal matter. Conservation Genetics Resources 5: 879–885. doi:10.1007/s12686-013-9900-1

Jusino M, Banik M, Palmer J, Wray A, Xiao L, Pelton E, Barber J, Kawahara A, Gratton C, Peery M, Lindner D (2017) An improved method for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous animals. Molecular Ecology Resources19(1): 176–190 https://doi.org/10.7287/peerj.preprints.3184v1

Kardynal KJ, Imlay TL (2022) Ecology and conservation of avian aerial insectivores. Canadian Journal of Zoology 100: 615–616. https://doi.org/10.1139/cjz-2022-0076

Kartzinel TR, Chen PA, Coverdale TC, Erickson DL, Kress WJ, Kuzmina ML, Rubenstein DI, Wang W, Pringle RM (2015) DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. Proceedings of the National Academy of Sciences of the United States of America 112: 8019–8024. https://doi.org/10.1073/pnas.1503283112

Lopes L, Fernandes A, Medeiros M, Marini M (2016) A classification scheme for avian diet types. Journal of Field Ornithology 87(3): 309–322. https://doi.org/10.1111/jofo.12158

Lourenço R, Pereira PF, Oliveira A, Ribeiro-Silva J, Figueiredo D, Rabaça JE, Mira A, Marques JT (2021) Effect of vineyard characteristics on the functional diversity of insectivorous birds as indicator of potential biocontrol services. Ecological Indicators 122: 107251. https://doi.org/10.1016/j.ecolind.2020.107251

Lourie SA, Tompkins DM (2000) The diets of Malaysian swiftlets. The Ibis 142: 596–602. https://doi.org/10.1111/j.1474-919X.2000.tb04459.x

Macarthur R, Levins R (1967) The Limiting Similarity, Convergence, and Divergence of Coexisting Species. American Naturalist 101: 377–385. https://doi.org/10.1086/282505

Mansor MS, Abdullah Halim MR, Abdullah N, Ramli R, Cranbrook E (2020) Barn Swallows *Hirundo rustica* in Peninsular Malaysia: Urban winter roost counts after 50 years, and dietary segregation from house-farmed swiftlets *Aerodramus* sp. The Raffles Bulletin of Zoology 68: 238–248. https://doi.org/10.26107/RBZ-2020-0021

Mansor MS, Rozali FZ, Davies S, Nor SM, Ramli R (2022) High-throughput sequencing reveals dietary segregation in Malaysian babblers. Current Zoology 68: 381–389. https://doi.org/10.1093/cz/zoab074

Michel NL, Smith AC, Clark RG, Morrissey CA, Hobson KA (2016) Differences in spatial synchrony and interspecific concordance inform guild-level population trends for aerial insectivorous birds. Ecography 39: 774–786. https://doi.org/10.1111/ecog.01798

Moradi HV, Zakaria M, Mohd AB, Yusof E (2009) Insectivorous birds and environmental factors across an edge-interior gradient in tropical rainforest of Malaysia. International Journal of Zoological Research 5: 27–41. https://doi.org/10.3923/ijzr.2009.27.41

Morse DH (1971) The Insectivorous Bird as an Adaptive Strategy. Annual Review of Ecology and Systematics 2: 177–200. https://doi.org/10.1146/annurev.es.02.110171.001141

Orłowski G, Karg J (2013) Diet breadth and overlap in three sympatric aerial insectivorous birds at the same location. Bird Study 60: 475–483. https://doi.org/10.1080/00063657.2013.839622

Otieno NE, Mukasi J (2023) Tropical insectivorous birds' predation patterns that promote forest–farmland trophic connectivity for integrated top–down pest biocontrol. Frontiers in Environmental Science 11: 1194267. https://doi.org/10.3389/fenvs.2023.1194267

Parker J, Kronauer DJC (2021) How ants shape biodiversity. Current Biology 31: R1208–R1214. https://doi.org/10.1016/j.cub.2021.08.015

Pianka ER (1974) Niche overlap and diffuse competition. Proceedings of the National Academy of Sciences of the United States of America 71: 2141–2145. https://doi.org/10.1073/pnas.71.5.2141

Pyke G, Pulliam H, Charnov E (1977) Optimal Foraging: A Selective Review of Theory and Tests. The Quarterly Review of Biology 52. https://doi.org/10.1086/409852

R Development Core Team (2020) RA language and environment for statistical computing, R Foundation for Statistical. Computing.

Razeng E, Watson DM (2015) Nutritional composition of the preferred prey of insectivorous birds: popularity reflects quality. Journal of Avian Biology 46: 89–96. https://doi.org/10.1111/jav.00475

Roslin T, Hardwick B, Novotny V, Petry WK, Andrew NR, Asmus A, Barrio IC, Basset Y, Boesing AL, Bonebrake TC, Cameron EK, Dáttilo W, Donoso DA, Drozd P, Gray CL, Hik DS, Hill SJ, Hopkins T, Huang S, Koane B, Laird-Hopkins B, Laukkanen L, Lewis OT, Milne S, Mwesige I, Nakamura A, Nell CS, Nichols E, Prokurat A, Sam K, Schmidt NM, Slade A, Slade V, Suchanková A, Teder T, van Nouhuys S, Vandvik V, Weissflog A, Zhukovich V, Slade EM (2017) Higher predation risk for insect prey at low latitudes and elevations. Science 356: 742–744. https://doi.org/10.1126/science.aaj1631

Sauer JR, Niven DK, Pardieck KL, Ziolkowski Jr DJ, Link WA (2017) Expanding the North American Breeding Bird Survey analysis to include additional species and regions. Journal of Fish and Wildlife Management 8: 154–172. https://doi.org/10.3996/102015-JFWM-109

Sekercioglu CH (2006) Increasing awareness of avian ecological function. Trends in Ecology & Evolution 21: 464–471. https://doi.org/10.1016/j.tree.2006.05.007

Shafie NJ, Anuar H, David G, Ahmad A, Abdullah MT (2023) Bird species composition, density and feeding guilds in contrasting lowland dipterocarp forests of Terengganu, Peninsular Malaysia. Tropical Ecology 64: 238–248. https://doi.org/10.1007/s42965-022-00267-5

Spiller KJ, Dettmers R (2019) Evidence for multiple drivers of aerial insectivore declines in North America. The Condor: Ornithological Applications 121(2): 1–13. https://doi.org/10.1093/condor/duz010

Symondson WOC (2002) Molecular identification of prey in predator diets. Molecular Ecology 11: 627–641. https://doi.org/10.1046/j.1365-294X.2002.01471.x

Turner A (2006) The Barn Swallow T. & AD Poyser. London, UK.

Whelan CJ (2001) Foliage structure influences foraging of insectivorous forest birds: an experimental study. Ecology 82: 219–231. https://doi.org/10.1890/0012-9658(2001)082[0219:FSIFOI]2.0.CO;2

Whelan CJ, Wenny DG, Marquis RJ (2008) Ecosystem Services Provided by Birds. Annals of the New York Academy of Sciences 1134: 25–60. https://doi.org/10.1196/annals.1439.003

Winkler DW, Luo MK, Rakhimberdiev E (2013) Temperature effects on food supply and chick mortality in tree swallows (Tachycineta bicolor). Oecologia 173: 129–138. https://doi.org/10.1007/s00442-013-2605-z

Zaccarelli N, Bolnick DI, Mancinelli G (2013) RInSp: An r package for the analysis of individual specialization in resource use. Methods in Ecology and Evolution 4: 1018–1023. https://doi.org/10.1111/2041-210X.12079

Zhang J, Ding Q, Huang J (2016) spaa: Species association analysis. R package version 02 2: 10.32614.

Supplementary material 1

Metrics to quantify the diet taxa abundance and details of the dietary preferences by four species of aerial insectivorous birds

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Link: https://doi.org/10.3897/natureconservation.59.152167.suppl1